ISSN 0494-3244

Тутун/Tobacco,Vol.63, N° 1-6,20-28, 2013 UDC: 633.71-152.61:631.8(497.2)

Original Scientific paper

QUALITY RESEARCH OF THE *KRUMOVGRAD 90* ORIENTAL TOBACCO CULTIVAR, GROWN IN THE *MESTA* TOBACCO REGION UNDER VARIED FERTILIZING CONDITIONS

NurettinTahsin, Tatyana Ortomarova, ShteliyanaKalinova

Agricultural University-Plovdiv, Bulgaria
E-mail: s_kalinova@yahoo.com

ABSTRACT

The quest for quality-specific raw tobacco aimed at obtaining homogenous lots preferred on the international market has led to the replacement of the typical *Nevrokop* tobacco cultivars by the *Krumovgrad* tobacco ecotypes and their growing in untypical regions such as the *Mesta* tobacco region. The mass entering of the*Krumovgrad* cultivars into almost all tobacco growing regions in Bulgaria has to a certain extent changed also the quality characteristics of the raw material. That fact posed the need of changing the traditional agro techniques applied for those types of tobacco in line with the peculiarities of the region and with a view to obtaining high quality raw tobacco. The traditional agro techniques for the *Nevrokop* tobaccos, used for the growing of the *Krumovgrad 90* in the *Mesta* tobacco region is most likely one of the reasons for obtaining raw tobacco inferior in its consumer and technological characteristics to that produced in its typical regions.

The present research proves the assertion that the use of the combined soil fertilizer – *Hydro* 4-21-21+ammonium nitrate and the combined soil fertilizer – *Cropcare* 12-22-8 + foliar spray – *Ferticare* 6-14-31 influence positively the quality of the *Krumovgrad 90* cultivar.

Key words: oriental tobacco, agro techniques, fertilizing, expert evaluation, quality

ИСТРАЖУВАЊЕ НА КВАЛИТЕТОТ НА ОРИЕНТАЛСКАТА СОРТА КРУМОВГРАД 90 ПРОИЗВЕДЕНА ВО ТУТУНОПРОИЗВОДНИОТ РЕОН МЕСТА ВО РАЗЛИЧНИ УСЛОВИ НА ЃУБРЕЊЕ

Потрагата по тутунска суровина со специфичен квалитет со цел да се добијат хомогенисерии какви што бара меѓународниот пазар доведе до замена на типичните тутунски сорти неврокоп со екотиповите од тутунот крумовград и до нивно одгледување во нетипични региони како што е тутунопроизводниот реон Места. Масовното навлегување на крумоврадските сорти во скоро сите тутунопроизводни реони во Бугарија до извесна мера ги измени и квалитативните својства на новиот материјал. Овој факт ја наметна потребата од промена на традиционалните агротехнички мерки кои се применуваат кај овие типови, за да одговараат на особеностите на реонот, со цел да се добие висококвалитетна тутунска суровина.

Примената на агротехничките мерки што се вообичаени за сортите неврокоп и во производството на Крумовград 90 во реонот на Места е, најверојатно, една од причините што добиената тутунска суовина по своите потрошувачки и технолошки својства е полоша во споредба со онаа што се произведува во својот типичен реон.

Со ова истражување се потврди дека примената на комбинираното почвено ѓубре Hydro 4 -21-21 + амониум нитрат и комбинираното почвено ѓубре Cropcare 12-22-8 + фолијарно прскање со Ferticare 6-14-31 има позитивно влијание врз квалитетот на сортата Крумовград 90.

Клучни зборови: ориенталски тутун, агротехнички мерки, ѓубрење, стручна проценка, квалитет

INTRODUCTION

It is mainly oriental tobacco that is grown in Bulgaria. Around 80% of its production is to be found in the Rila-and-Pirin and the Rhodopi-and-Trakia districts (Slavova, Drachev, 2004). The tobacco ecotypes, marked by their specific technological features, are set up in differentiated regions under the impact of mainly two basic facts – the local soil and climatic conditions. Tobacco grown on unsuitable soils, loses to some extent its type (Georgiev, 2005), apart from its quality. The Krumovgrad 90 cultivar, grown in the Mesta tobacco region does not realize its biological potential and is inferior in biological and economic traits to the same cultivar, grown in the typical Bashibalijski region. In the latter region the Krumovgrad 90 cultivar forms thicker, larger and richer leaves of dominant red hues featuring a raw material of a pleasant flavour, specific aroma and medium to above- medium strength (Nikolova, Nikolov, Drachev, 2007; Drachev, Nicolova, 2006; Nicolova, 2007). Asto thequality of the obtained raw leaves expressed in grade percentages in the Mesta tobacco region, the first grade percentage is 34% whereas in the Bashibalijski region it exeeds45% (Georgiev, 2005; Mladenov, 2006). The technological investigations on the quality of the cured tobacco of the Krumovgrad 90 cultivar, grown in the Mesta tobacco region and on the tobacco typical of the Bashibalijski region, showed that the crude tobacco obtained from Krumovgrad 90 grown in the regions and sub-regions of the Nevrokop ecotype is inferior to the raw material from the typical Bashibalijski region in relation to all quality features. The detected differences are in the exterior quality traits, chemical content and smoking properties (Drachev, Nicolova, 2006).

Suitable agro ecological conditions are a necessary prerequisite for the full and adequate unfolding of the genotype potential. Under intensive production terms tobacco realizes its maximum biological potential only when applying the right and up-to-date agro techniques (Zdraveska, 2006; Zdraveska et all., 2007; Pelivanoska 2008; Trajкoskі, Pelivanoska, 2003). Fertilization and irrigation are the most important of the complex agro technical measures, exerting a direct influence on the biological and economic features of raw tobacco (Hristoskiet all., 2007). Only when matching the appropriate cultivars, soils and technology with the producers' experience is it possible to obtain high-quality tobacco and profitable production (Georgiev, 2005).

The aim of the present research was to study the exterior features of the Krumovgrad 90 cultivar, grown in the untypical Mestatobacco region with different variants of fertilizer application.

MATERIAL AND METHODS

The object of investigation was the *Krumovgrad 90* oriental tobacco cultivar grown in the *Mesta* tobacco region. The trials were carried out in 2007 and 2008 in two sub-regions, representative of the *Mesta* tobacco region: the *Polski* sub-region /valley-type/ and the *Yaka* sub-region. The *Polski* sub-region /valley-type/ comprised the micro-regions of *Borovo* and *Banichan* while the *Yaka* sub-region included the micro-regions of *Lazhnitsa*, *Kornitsa* and *Breznitsa*. The fertilizer rates in kg/da of active substance (Table 1) were fixed on the basis of preliminary soil analysis. Both single and combined fertilizers were used for the purpose of the research in the following variants: variant 1 /control/– non-fertilized; variant 2 – applying ammonium nitrate, triple superphosphate, potassium sulphate; variant 3 – using the combined soil fertilizer – *Hydro* 4-21-21+ammonium nitrate; variant 4 – applying the combined soil fertilizer – *Cropcare* 12-22-8 + foliar spray – *Ferticare* 6-14-31 in a concentration of 0.4%. Thefertilizers were incorporated in the rows at the stage of transplanting. The foliar spray was applied three times at an interval of 10 days, the first application done at the beginning of the intensive growth stage. The tobacco growing was implemented in compliance with the technology, well-recognized at the time of the research,for oriental tobacco cultivation consistent with the agro technical recommendations of the author of the cultivar – *Manolov* (1979). The deadline for transplanting was within the agro technical period for Southern Bulgaria (30th April –20th May). The quality rating of the cured tobacco was done by using the *expertise* method directly comparing the samples.

Table 1. Fertilizer rates in kg/da active substance

Sub-region*Polski* /valley-type/		Sub-region *Yaka*		
Micro-region*Borovo*	Micro-region *Banichan*	Micro-region *Kornitsa*	Micro-region *Lazhnitsa*	Micro-region *Breznitsa*
N – 3.0	N – 3.8	N – 3.5	N – 3.5	N – 2.0
P – 6.0	P – 8.0	P – 7.0	P – 7.0	P – 6.0
K – 6.0	K – 7.0	K – 7.0	K – 7.0	K – 5.0

RESULTS AND DISCUSSION

The expert evaluation plays a significant role in the quality grading of oriental tobacco taking into consideration its peculiarities as both a consumer product and commodity meant mostly for export. It is done on the basis of the exterior quality traits, their peculiarities and degrees of manifestation in the varied leaf categories.

The expert evaluation of tobacco is a must at all stages, from its purchase to its inclusion in separate trading batches andpresence in the cigarette blends.

In the present research the method of *profile description* was used in carrying out the expert evaluation with a view to achieving a more definitive characterization of the exterior quality traits of the separate samples. The degree of manifestation of each quality trait was determined by accrediting a certain number of points.The final evaluation was the sum total of the pointsand the listingaccording to the sum. The sample with the fewest pointswas the bestand was given rank 1.

The results of the expert evaluation of the tobaccos grown in the *Polski* sub-region /valley-type/ and the *Yaka* sub-region during the 2007 yield year are given in Table 4. The data show that when grading the tobaccos of the 4 variants of fertilizer application in the 5 micro-regions, the highest quality of the exterior traits was observed in the control (the non-fertilized variant), followed by variant 3.

The evaluation for the separate sub-regionswas as follows:

Sub-region *Polski* /valley-type/ (Table 2): No significant differences were detected in the compared samplesin the *Banichan*micro-region. The control ranked first, followed by variant 3, variants 4 and 2 coming last.

In the *Borovo* micro-region the control showed the best exterior quality traits. There was no credible difference between variants 4 and 3, ranking second and third respectively, since the differences in the sums of the pointsof the two samples were insignificant. The variant 2 sample was inferior to both the control and the rest of the tested variants regarding the exterior quality traits.

Sub-region *Yaka*(Table 2)**:**In reference tothe *Breznitsa* micro-region: The obtained results showed variant 3 to possess the best exterior quality traits, followed by the control and variant 4 ranking in the last place.

In the *Kornitsa* micro-region the grading of the tobaccos grown with different fertilizer application variants was as follows: the control demonstrated the best exterior quality traits, variants 2 and 4 coming next and variant 3 in the last place.

In the *Lazhnitsa* micro-region the control sample had the best exterior quality traits, followed by variants 3 and 4, variant 2 coming in the last place. It should be noted, however, that the differences between variants 3 and 4 were insignificant.

The results of the expert evaluation of the tobaccos grown in the *Polski* sub-region /valley-type/ and the *Yaka* sub-region during the 2008-yield year are given in Table 3.

The obtained outcomes show that for the 4 variants of fertilizer application in the 5 micro-regions during 2008, the highest quality of the exterior traits was observed in variant 3 from the *Polski* sub-region /valley-type/ and variant 4 from the *Yaka* sub-region.

The expert evaluation for the micro-regionsand fertilizer variants was as follows:

Sub-region *Polski* /valley-type/ (Table 3):
The *Banichan* micro-region demonstrated the best tobacco quality in the control. No significant difference was observed among the rest of the variants. The sums of the pointswere quite close, especially in variants 2 and 3, standing in third and fourth place respectively.

In micro-region *Borovo* variant 3 manifested the best traits, variant 2 having the lowest values.

Sub-region *Yaka* (Table3)
In the *Breznitsa* micro-region the control showed the best quality traits, followed by variant 4. The differences in quality among the variants were most sharply expressed in that specific micro-region.

In the *Kornitsa* micro-region variant 4 was of the best traits. The control conceded considerably as to the number of points(31.2). The two other variants possessed traits similar to those of the control.

In the *Lazhnitsa* micro-region the positioning of the variants was as follows: variant 4 demonstrated the best quality traits in the expert evaluation, variant 2coming next. The quality traits values were lower, as compared with the control, only in variant 3. Variant 4 ranked either first or second, immediately after the control, in all micro-regions at the expert evaluation of tobaccos for the 2008-yield year.

Table 2.Expert evaluation of the tobaccos from the *Polski* sub-region /valley-type/and *Yaka* sub-region for the 2007 yield

Expert evaluation of the tobaccos from the *Polski* sub-region /valley-type/for the 2007 yield																		
		Indicators																
Variant	Micro-region	1	2	3	4	5	6	7	8	9	10	11	12	13	∑ 1-13	Grading	$\bar{X}$	Grading
	Banichan	1	1	2	1	1	2	1	1.5	2	3	3	1	1	20.50	1		
1*	Borovo	1.5	1.75	2	1	1	2	1	1	2	3.5	3	1	1	21.75	1	21.12	1
	Banichan	1	1	2	1	1	2	2	1.5	2	3	3	1	2.5	23.00	4		
2*	Borovo	1	1.75	2.5	1	2	2	2	3	2	3	3	1	1	25.25	4	24.12	4
	Banichan	1	1	2	1.5	1.5	2	1	1.5	2	3	3	1	1	21.50	2		
3*	Borovo	1.5	1.75	2	1	1	2	1	3	2	3.5	3	1	1	23.75	3	22.62	2
	Banichan	1	1	2.5	1.5	1	3	3	1.5	2	1	3	1	1	22.50	3		
4*	Borovo	1	1	2	1.5	2	2	2	1	2	3.5	3	1	1	23.00	2	22.75	3
Expert evaluation of the tobaccos from the *Yaka* sub-region for the 2007 yield																		
		Indicators																
Variant	Micro-region	1	2	3	4	5	6	7	8	9	10	11	12	13	∑ 1-13	Grading	$\bar{X}$	Grading
	Breznitsa	2	1	2.5	2	2	2	3	1	2	3	3	1	1	25.50	2		
	Kornitsa	1.5	1	1.5	1	1	2	2	1	2	2.5	3	1	1	20.50	1		
1*	Lazhnitsa	1	1	1.5	1.5	1	2	1.5	1.5	1	1.5	2	1	1	17.50	1	21.17	1
	Breznitsa	1.5	2	2.5	3.5	3	3	3	3.5	2	4	3	1	1	33.00	3		
	Kornitsa	1.5	1	2	1.5	2	2	2	2	1.5	3	3	1	1	23.50	2		
2*	Lazhnitsa	1	1.5	2.5	1.5	1	2	2.5	3.5	2.5	3	3	1	1	26.00	4	27.50	3
	Breznitsa	1	1.75	2.5	2	2	2	2.5	1	1.5	3	3	1	1	24.25	1		
	Kornitsa	2	2.5	2.5	2.5	3	3	3	4.5	4	4.5	3	1	1	36.50	4		
3*	Lazhnitsa	1	1.5	2	2	2	2	2	1.5	1	1.5	2	1	1	20.50	2	27.08	2
	Breznitsa	1.5	2.5	2.5	2	3	3	3	4	4	4.5	4	1	2.5	37.50	4		
	Kornitsa	1	1	2.5	2.5	2.5	3	3.5	3.5	2	3	3	1	1	29.50	3		
4*	Lazhnitsa	1	1	1.5	1.5	1.5	2	2	1	1	3.5	3	1	1	21.00	3	29.33	4

Table 3.Expert evaluation of the tobaccos from the *Polski* sub-region /valley-type/and *Yaka* sub-region for the 2008 yield

Expert evaluation of the tobaccos from the *Polski* sub-region /valley-type/for the 2008 yield																		
Variant	Micro-region	Indicators																
		1	2	3	4	5	6	7	8	9	10	11	12	13	∑ 1-13	Grading	$\bar{X}$	Grading
	Banichan	1.8	1	2	2	1	2.5	1.8	1	1.8	1	3	1	1	20.90	1		
1*	Borovo	2	1	2	1.5	1	2.5	2	1.5	2	3	3	1	1	23.50	3	22.20	2
	Banichan	1.5	1	2	1	1	3	2	1.5	2	3.5	3	1	1	23.50	3		
2*	Borovo	2	1	2.5	1.5	1.5	2.8	2.5	1	2	3	3	1	1	24.80	4	24.10	4
	Banichan	1.5	1	2	1	1	2.5	3	1.8	2	3	3	1	1	23.80	4		
3*	Borovo	1.7	1	17	1.3	1	2	1.2	1	1.5	1.5	2	1	1	17.90	1	20.80	1
	Banichan	1	1	2	1.5	1	2.5	2.5	1	2	3	3	1	1	22.50	2		
4*	Borovo	2	1	2.2	1.8	2	2	2	1	1.7	3	2.5	1	1	23.20	2	22.80	3
Expert evaluation of the tobaccos from the *Yaka* sub-region for the 2008 yield																		
Variant	Micro-region	Indicators																
		1	2	3	4	5	6	7	8	9	10	11	12	13	∑ 1-13	Grading	$\bar{X}$	Grading
	Breznitsa	1.5	1	2.5	2	2	3	3	1	2	3.5	3	1	1	26.50	1		
	Kornitsa	1.5	1.7	2.5	2	2	3	4	2	3	4.5	3	1	1	31.20	4		
1*	Lazhnitsa	1.5	1	2	2	2	3	2	1	2	4.5	3	1	1	26.00	3	27.90	3
	Breznitsa	2	1	3	3	3	3	4	2	2	4.5	3	1	1	32.50	3		
	Kornitsa	1.7	1	2	2	2	2.5	2.5	1	2	3.5	3	1	1	25.20	3		
2*	Lazhnitsa	2	1	2	2	2	3	2	1	2	3.5	3	1	1	25.50	2	27.70	2
	Breznitsa	2	1	3	4	3	3	4	4	3.7	3	3	1	1	35.70	4		
	Kornitsa	2	1	2	1.5	2	2.5	2	1	2	3.5	3	1	1	24.50	2		
3*	Lazhnitsa	1.5	1	2.2	2	2	3	3	1	2	4.5	3	1	1	27.20	4	29.10	4
	Breznitsa	2	1.7	2.2	2.5	2	3	2.5	2	2	3.5	3	1	1	28.40	2		
	Kornitsa	1.3	1	2	1.8	1	3	3	1	2	3	3	1	1	24.10	1		
4*	Lazhnitsa	1.5	1	2	2	2	2.5	2	1	2	3.5	3	1	1	24.50	1	25.70	1

1* - non-fertilized variant (control); 2* - application of ammonium nitrate, triple superphosphate and potassium sulphate; 3* - using a combined soil fertilizer and a supplement of ammonium nitrate; 4*- a combination of a soil fertilizer and foliar spray.
1-cropping (pulling) area of the stalk; 2 –ripeness; 3 –richness; 4 –leaf structure; 5 –elasticity; 6 –resin content;7 –aroma; 8 –basic colour; 9 – basic colourintensity; 10 – hues; 11 – gloss; 12 – injuries caused by diseases and pests; 13 – injuries caused in the curing process.

It is obvious from the mean data (Table 4) for the expert evaluation of the tested tobaccos that the control exhibited the best exterior quality traits in the Polski sub-region /valley-type/, followed by variant 3, the differences in the ranks being insignificant – 21.66 forvariant 1 and 21.71 for variant 3.
In the Yaka sub-region variant 4 demonstrated the best exterior quality traits out of the fertilized variants – 27.51.

Table 4.Expert evaluation of tobaccosin 2007 – 2008

Expert evaluation of tobaccosin 2007 – 2008								
	Variants of fertilizer application							
	1*		2*		3*		4*	
Sub-region	Average	Grading	Average	Grading	Average	Grading	Average	Grading
Polski/valley-type/	21.66	1	24.11	4	21.71	2	22.77	3
Yaka	24.53	1	27.60	3	28.09	4	27.51	2

CONCLUSIONS

Based on the data about the impact of the different kinds of fertilizers on the exterior quality traits of the tobaccos grown in the Polski sub-region /valley-type/ and the Yaka sub-region, the following conclusions could be drawn:
Under the conditions of the Polski sub-region /valley-type/, out of the variants with fertilizer application, variant 3 exhibited the best exterior quality traits /when using the combined soil fertilizer – Hydro 4-21-21+ammonium nitrate/.
Under the conditions of the Yaka sub-region, out of the variants with fertilizer application, variant 4 exhibited the best exterior quality traits /when applying the combined soil fertilizer – Cropcare 12-22-8 + foliar spray – Ferticare 6-14-31/.

REFERENCES

1. GeorgievHr., 2005, Dependencies between the leaf sizes and the technologies in oriental tobacco, Bulgarian Tobacco, 3,13-14.

2. Zdraveska N., 2006, Agrochemical characteristics of the soils in the Ovchepol tobacco growing region, Tobacco, vol. 56, № 9-10:173-182.

3. Zdraveska N. et al. 2007, Agrochemical characteristics of the soils in the Veleshki tobacco growing region, Tobacco, vol. 57, № 1-2: 21-32.

4. ManolovA.,1979, Characteristics of tobacco originating from Krumovgrad, Bulgarian Tobacco, vol. 8,12-14.

5. MladenovE., 2006, Annual Reports ofETS – GotseDelchev.

6. NikolovaV., N.Nikolov, D.Drachev, 2007, A complex technological assessment of oriental tobacco from the GotseDelchev region, scientific journal of agriculture and forestry *Ecology and the Future*, IV(2), 30-33.

7. Pelivanoska V., 2008, Fertility of the soils for tobacco growing in the region of St. Nikola, Tobacco, vol. 58, №7-8: 166-178.

8. SlavovaY., D. Drachev, 2004, Elaboration of the cultivar regional distribution – a market requirement for oriental tobacco, Agricultural Economics and Management, 3

9. TrajkoskiJ., V. Pelivanoska, 2003, Fertility of the soils for tobacco growing in the region ofRadovish, Tobacco, vol.53, № 9-10:284-302.

10. HristoskiZh. et al., 2007, Influence of fertilizing and irrigation on the yield and quality of some tobacco cultivars of the Prilep type, Tobacco, vol.57, №3: 62-71.

11. Drachev D.,V.Nicolova, 2006, Technological study on the quality of tobacco varieties Dzhebel K81 and Krumovgrad 90 grown in inconventional regions, Tobacco, 56 (7-8):149-158.

12. Nicolova V., 2007, Technological investigation on quality and possibilities for widening the production of marked demanded oriental type tobaccos. Message II: Technological investigation on Krumovgrad 90, Bulgarian Journal of Agricultural Science, 13 (1):63-67.

ISSN 0494-3244

Тутун / Tobacco,Vol.63, N° 1-6,29-35, 2013 UDC:633.71-116.4:575.22(497.775)"2010/211"

Original Scientific paper

THE INFLUENCE OF GENOTYPE ON YIELD, QUALITY AND ECONOMIC EFFECTS OF BURLEY TOBACCO

Ilija Risteski, Karolina Kočoska

University St. Climent Ohridski - Bitola - Scientific Tobacco Institute - Prilep, Republic of Macedonia
e-mail: ilija.r@t-home.mk

ABSTRACT

During 2010 and 2011 investigations with 6 Burley tobacco varieties and lines were made in order to study their influence on yield and quality of the obtained raw material, and to evaluate their economic effects. The results of investigations showed absolute dominance of variety Pelagonec CMS F_1 and line B-98/N CMS F_9 over the other varieties, which was statistically confirmed. From a practical point of view, these results can be a good guideline to tobacco growers in selection of tobacco variety.

Key words: tobacco, variety, Burley, yields, economic effects

ВЛИЈАНИЕТО НА ГЕНОТИПОТ ВРЗ ПРИНОСОТ, КВАЛИТЕТОТ И ЕКОНОМСКИТЕ ЕФЕКТИ КАЈ ТУТУНОТ ОД ТИПОТ БЕРЛЕЈ

Во текот на 2010 и 2011 година во испитувањата беа вклучени 6 берлејски сорти и линии тутун со цел да се испита нивното влијание врз приносот и квалитетот на добиената суровина и економскиот ефект што го даваат истите. Добиените резултати од испитуваните својства покажаа апсолутна доминација на сортата Пелагонец ЦМС F_1 и линијата Б-98/N ЦМС F_9 над другите сорти што и статистички беше потврдено. Од практичен аспект овие резултати во иднина можат да бидат добра смерница при изборот на сорта од страна на примарните производители.

Клучни зборови: тутун, сорта, берлеј, приноси, економски ефект.

INTRODUCTION

Raw material of Burley and Virginia tobaccos participate in the composition of blend cigarettes with about 80%. The first steps towards introducing the type Burley in the Republic of Macedonia were made by Rudolf Gornik, who reported (1953) that this tobacco can be successfully cultivated only in rich soils and humid climate with frequent rainfalls. In early 70ies efforts were made towards creating a variety which will prove to be the best in most of the properties, especially in yield and quality. In that period, the main representative of this type of tobacco in the Republic of Macedonia was the Croatian male sterile variety Chulinec. Later on, male sterile varieties Burley B-96/85 CMS F_1, Burley 1 CMS F_1, B-2/93 CMS F1 and

Pelagonec CMS F1 were created in Tobacco Institute - Prilep. These varieties were a satisfactory substitute for the variety Chulinec, and some of them found their way beyond the borders of Macedonia. The fact that there is no ideal variety created once and for all, but that some variety at a given moment is better than the others, motivated the breeders of Tobacco Institute - Prilep to create new varieties (genotypes) with improved characters, i.e. with higher yields and quality. Since these characters are governed by the genetic structure, parents in which these characters are predominant are used in the process of hybridization. This process is exclusively intervarietal and is conducted with the aim to obtain male sterile hybrid varieties. The best of them are tested in field, in comparative trials with other standard varieties (domestic and foreign) for a period of at least two years. If they show better results than the standard, they are submitted to the State Variety Commission for recognition. This paper will present the results of investigations on yield and quality of the raw material obtained from the varieties and lines represented in the research.

MATERIAL AND METHODS

The investigations were carried out in the Experimental field of Tobacco Institute - Prilep during 2010 and 2011, on coluvial-alluvial soil. It included three introduced fertile varieties of Burley tobacco (B-21 from USA, Banquet 21 from Zimbabwe and B- 1317 from Bulgaria), the male sterile hybrid variety Pelagonec CMS F_1 and lines B-98/N CMS F_9, B-136/07. The variety B-21 was used as a check. Autumn ploughing was carried out at about 40 cm depth and prior to spring ploughing, the soil was fertilized with 300 kg/ha NPK 8: 22: 20. Before transplanting, the soil was treated with herbicide and, immediately after, it was incorporated into the soil by harrowing. The trial was set up in randomized blocks with 4 replications, at 90 × 50 cm spacing. Two hoeings of tobacco were applied, followed by addition of 5g of 26% CAN. A few additional irrigations during the growing period were applied when necessary. After harvest and stringing, tobacco was yellowed and air-cured in special curing barns for Burley tobacco. Qualitative estimation of dried tobacco was made according to the Rules for standard measurements of quality of leaf tobacco of the type Burley. Corrected yield per stalk and per hectare was estimated by the method of Rimker and gross income (denars/ha) was assessed when the yield per hectare was multiplied with the average price per 1 kg of raw tobacco. Statistical processing of data was performed using the analysis of variance technique.

RESULTS AND DISCUSSION

The yield of tobacco, as in many other crops, is affected by the genotype, as well as genotype : environment interaction. Tobacco yield as quantitative character is in close correlation with leaf number, size and thickness. There are differences between the varieties of the same type, but it still must be typical for that type. Budim T. (1988) reports that the average yield of Burley tobacco in Zimbabwe in the period 1980-1985 ranged from 1202 to 1760 kg/ha. The development of selection of this tobacco in the world resulted in creation of new genotypes that produce significantly higher yields, without negative effects on quality. Stoyanov Boris and Apostolova

Elena (1999) reported that the yields of B-1317 variety in some parts of Bulgaria can reach up to 3380 kg / ha.
According to Djulgerski Yovko (2009), the yield of Burley tobacco should not be lower than 3500 kg/ha. Ilija Risteski and Karolina Kocoska (2012) reported that Burley varieties created in Tobacco Institute- Prilep gave a yield of 3500-4500 kg/ha. The yield of this tobacco type is strongly affected by agrotechnical measures applied. Pelivanoska V. (2001) reported that by different variants of fertilization and irrigation, the yields of B-2/93 CMS F1 varieties in the region Ohrid-Struga can reach up to 6000 kg/ha.

Yield per stalk (g/stalk)

Data on variations of yields per stalk in varieties and lines investigated in the trial are presented in Table 1.

Table 1 Corrected yield, g/stalk

Varieties	Years	Average yield, g/stalk	Average 2010/11	Differences from the average		Range
				Absolute	Relative	
B-21	2010	123.5	122.3	/	100.00	3
	2011	121.1				
B-1317	2010	118.8	119.7	- 2.6	97.84	5
	2011	120.6				
Banquet 21	2010	122.3	118.9	- 3.4	97.22	6
	2011	115.9				
B-136/07	2010	121.4	121.6	- 0.7	99.42	4
	2011	121.9				
B-98/N CMS F_9	2010	158.3^{++}	166.0	+ 43.7	135.73	2
	2011	173.7^{++}				
Pelagonec CMS F_1	2010	168.3^{++}	176.0	+ 53.7	143.91	1
	2011	183.8^{++}				

2010	2011
LSD 5% = 21.11 g/stalk +	LSD 5% = 14.97 g/stalk +
1% = 29. 23 g/stalk ++	1% = 20.74 g/stalk ++

According to the above data, the highest average yield per stalk of 176.0 g was recorded in the variety Pelagonec CMS F_1. It is 53.7 g or 43.91% higher compared to the check variety B-21, which average yield was 122.3g/stalk. The lowest average yield of 118.9 g/stalk was achieved in the variety Banquet 21, and it is 3.4 grams or 2.78% less than that of the check variety. In the other varieties and lines, the average yield ranges from 119.7 g/stalk in variety B-1317 to 166.0 g/stalk in line B-98/N CMS F_9. In both years of investigations, statistically significant differences at a level of 1% compared to the check were estimated in the variety Pelagonec CMS F_1 and line B-98/N CMS F9.

Yield per hectare (kg / ha)

The yield per hectare is closely related with quality per stalk. The combination of these two characters is a more expressive indicator in assessing the economic value of the genotype. Beside the impact of the variety, this character is also affected by some agro-technical measures. Janos Berenji and Miroslava Nikolic (1996) found that topping of the inflorescence, combined with sucker control in Burley tobacco can result in 28% yield increase per hectare.

Table 2 Corrected yield per hectare (kg/ha)

Varieties	Years	Average yield, kg/ha	Average 2010/11	Differences from the average: Absolute	Relative	Range
B-21	2010	2744	2717	/	100.00	3
	2011	2691				
B-1317	2010	2641	2661	- 56	97.93	5
	2011	2681				
Banquet 21	2010	2641	2608	- 109	95.99	6
	2011	2575				
B-136/07	2010	2698	2704	- 13	99.52	4
	2011	2710				
B-98/N CMS F_9	2010	3520^{++}	3692	+ 975	135.88	2
	2011	3864^{++}				
Pelagonec CMS F_1	2010	3740^{++}	3912	+ 1195	143.98	1
	2011	4085^{++}				

2010	2011
LSD 5% = 466.56 kg/ha+	LSD 5% = 332.72 kg/ha +
1% = 646.17 kg/ha++	1% = 460.82 kg/ha ++

According to the data presented in Table 2, the highest average yield per hectare of 3912 kg was recorded in the variety Pelagonec CMS F_1, which is 1195 kg (43.98%) higher compared to the check variety B-21, which average yield was 2717 kg/ha. The lowest average yield per hectare of 2608 kg was obtained in variety Banquet 21. In other varieties and lines, the average yield per hectare ranged from 2661 kg in the variety B-1317 to 3692 kg in line B-98 / N CMS F_9 in both years of investigations (2010 and 2011).

Average price, denars / kg

The average price is, in fact, an indicator of quality of the obtained tobacco raw expressed in monetary value. However, the quality of tobacco is a very complex concept, affected by many mutually dependent factors and influences. So this indicator is only the beginning of a series of procedures for estimation of tobacco quality (physical and chemical properties, degustation, etc.). The quality of tobacco raw and the average price depend on a number of adequately performed cultural practices in field, in the time of harvest, yellowing, curing, etc. Data on the average price per 1 kg of dry tobacco in investigated varieties and lines are presented in Table 3.

Table 3 Average price denars/ kg

Varieties	Years	Average price, denars/kg	Average 2010/11	Differences from the average: Absolute	Differences from the average: Relative	Range
B-21	2010	30.98	32.63	/	100.00	5
	2011	34.29				
B-1317	2010	36.27	34.57	+ 1.94	105.94	3
	2011	32.87				
Banquet 21	2010	28.10	28.77	- 3.86	88.17	6
	2011	29.44				
B-136/07	2010	36.27	32.74	+0.11	100.33	4
	2011	29.21				
B-98/N CMS F_9	2010	37.22^{++}	40.38	+7.75	123.75	2
	2011	43.55^{++}				
Pelagonec CMS F_1	2010	44.67^{++}	44.79	+12.16	137.26	1
	2011	44.92^{++}				

2010	2011
LSD 5% = 2.27 kg/ha+	LSD 5% = 3.15 kg/ha +
1% = 3.14 kg/ha++	1% = 4..36 kg/ha ++

Data from the table show that the highest average price of 44.79 denars / kg was obtained with variety Pelagonec CMS F_1, and that is 12.16 denars / kg or 37.26% higher compared to the check variety with an average price of 32.98 denars / kg.

The lowest quality and the lowest average price of only 28.77 denars / kg was recorded in the variety Banquet 21. In other varieties and lines, the average price ranges from 32.74 denars/kg in line B-136/07 to 40.38 day / kg in line B-98/N CMS F_9. Statistical differences at 1% significance level compared to the check in both years of investigation were obtained in the variety Pelagonec CMS F_1 and the line B-98 / N CMS F_9.

Gross income, denars/ha

The most important factors in the formation of this character are the average yield per hectare and the average price of 1 kg raw tobacco, i.e. it represents the yield and quality achieved by the varieties and lines investigated in the trial.

Table 4 Gross income (economic effect, denars /ha)

Varieties	Years	Gross income, denars/ha	Average 2010/11	Differences from the average: Absolute	Relative	Range
B-21	2010	85 568	88 588	/	100.00	5
	2011	91 609				
B-1317	2010	98 094	93 212	+ 4 624	105.22	3
	2011	88 330				
Banquet 21	2010	76 260	79 530	- 9 058	89.77	6
	2011	82 801				
B-136/07	2010	98 094	88 738	+150	100.17	4
	2011	79 383				
B-98/N CMS F_9	2010	131 596^{++}	150 438	+61 850	169.91	2
	2011	169 281^{++}				
Pelagonec CMS F_1	2010	167 613^{++}	175 528	+86 940	198.0	1
	2011	183 443^{++}				

2010	2011
LSD 5% = 20 843 kg/ha+	LSD 5% = 14 186 kg/ha +
1% = 28 867kg/ha++	1% = 19 647 kg/ha ++

According to the above data, the highest average gross income of 175 528 denars/ha was recorded in the variety Pelagonec CMS F_1, which is 86 940 denars/ha, i.e. 98.13% higher than the check variety B-21, which achieved 88 588 denars/ha. This indicator has the lowest value in the variety Banquet 21 (79 530 denars / ha). In other varieties and lines, the gross income ranges from 88 738 denars/ha in line B-136/07 to 150 438 denars/ha in line B-98/N CMS F_9. Statistically significant differences at 1% level compared to the check variety were estimated in variety Pelagonec CMS F1 and line B-98/N CMS F_9 in both years of investigation (2010 and 2011).

CONCLUSIONS

Based on the data obtained during the investigation, the following conclusions can be drawn:

- All varieties and lines included in the field trial developed under the same conditions of growing, but in the end they showed different results, as a product of various reactions of the varieties dictated by their genetic structure.
- The yields per stalk and per hectare were the highest in the variety Pelagonec CMS F_1 (176.0 g/stalk and 3912 kg/ha), and the lowest in the variety Banquet 21 (118.9 g/stalk and 2608 kg/ha).
- The average price for 1 kg of raw tobacco was the highest in the variety Pelagonec CMS F_1 (44.79 denars/kg) and the lowest in Banquet 21 (28.77 denars /kg).

- The gross income was the highest in the variety Pelagonec CMS F_1 (175 528 denars/ha), and the lowest in the variety Banquet 21 (79 530 denars/ha).
- Data obtained from the investigations show absolute dominance of the variety Pelagonec CMS F_1 and line B-98/N CMS F_9 over the other varieties and lines, which has been confirmed statistically.
- The obtained results lead to a conclusion that the variety has a very big influence on some productional characters. For this reason, in selection of varieties it is very important to have a deep knowledge of their properties.

REFERENCES

1. Berenji J., Nikolić M., (1996) Uticaj zalamanja cvsti i uklanjanje zaperaka na prinos i kvalitet lista duvana tipa Burley. Тутун/Tobacco Vol. 46, N0 1-6. Институт за тутун – Прилеп
2. Budin T. (1988) – Dostignuća i organizacije ustrojstva privrede Zimbabwea – Тутун / Tobacco Vol. 48, N^0 1-2. Институт за тутун – Прилеп
3. Gornik R. (1985) – Proizvodnja duhana tipa burley-Zagreb.
4. Дюлгерски Й., 2009. Сортов идеал при тютюн тип Бърлей - Българси тютюн, бр. 6/2009 стр. 16-18, София
5. Пеливаноска В. et al. (2001). Влијание на агротехничките услови и применетата агротехника врз квалитетните карактеристики на типот берлеј во охридско – струшкиот производен реон – Извештај за проектна задача на научно истражувачката работа во 2001 година ЈНУ – Институт за тутун Прилеп.
6. Risteski I., Kočoska K., (2012) Results of broadleaf tobacco breeding in Scientific Tobacco Institute – Prilep, International Symposium for agriculture and food. 12-14 December 2012, Skopje, Republic of Macedonia.
7. Стоанов Б., Апостолова Е., (1999). Нов сорт Бърлей 1317 – Български тютюн 6/1999 – Пловдив.

ISSN 0494-3244

Тутун/Tobacco,Vol.63, N⁰1-6, 36-46, 2013 UDC: 632.952.:633.71-248.843(497.7)
633.71-248.843:632.952 (497.7)

Original Scientific paper

THE EFFECTIVENESS OF FUNGICIDES IN THE CONTROL OF ALTERNARIA ALTERNATA DEPENDING ON THEIR IMPACT ON PATHOGEN BIOLOGY

Biljana Gveroska

University "St. Kliment Ohridski" - Bitola
Scientific Tobacco Institute-Prilep, Kicevski pat bb, 7500 Prilep
e-mail: gveroska@t-home.mk

ABSTRACT

Investigations were carried out to study the effectiveness of some fungicides in the control of pathogenic fungus Alternaria alternata in laboratory conditions. Their impact on conidial germination and growth in solid and in liquid media was studied in this paper.

The highest reducing effect on conidia germination was obtained by application of fungicides Folicur EW-250, Score 250-EC and Acrobat MZ – with only 2.97%, 11.60% and 15.19% germinated conidia. The poorest results were obtained with Dithane M-45 and Antracol WP-70.

Fungal growth in solid and liquid media was prevented by application of Folicur EW-250. With application of Score 250 EC it reached only 8.30 mm and with Baycor WP 25 -14.70 mm

However, high yield of dry biomass was obtained with Baycor WP 25 (140.00 mg), while with Dithane M-45 it was 20.00 mg.

According to the investigations, the effectiveness of fungicides varies in different environmental conditions, depending on its impact on the biology of the pathogen.

Fungicides Folicur EW-250 (0.1%), Score 250-EC (0.05%) and Acrobat MZ (0.25%) showed the best results in control of the pathogen.

Keywords: Alternaria alternata, fungicide, inhibition, biology

ЕФИКАНОСТ НА ФУНГИЦИДИТЕ ЗА СУЗБИВАЊЕ НА *ALTERNARIA ALTERNATA* ВО ЗАВИСНОСТ ОД НИВНОТО ВЛИЈАНИЕ ВРЗ БИОЛОГИЈАТА НА ПАТОГЕНОТ

Истражувањата беа извршени со цел да се испита ефикасноста на фунгицидите за сузбивање на патогената габа Alternaria alternata во лабораториски услови. Беше испитувано влијанието врз 'ртењето на конидиите како и развојот врз цврста и течна подлога.

Најголем редуцирачки ефект врз 'ртењето на конидиите покажаа фунгицидите Folicur EW-250, Score 250-EC и Acrobat MZ со само 2.97%, 11.60 % и 15.19% из'ртени конидии. Најслаби резултати имаа Dithane M-45 и Antracol WP-70.

Габата не се развиваше на цврста и течна подлога со препаратот Folicur EW-250. Кај Score 250-EC достигна само 8.30 mm а кај Baycor WP 25 -14.70 mm

Но, приносот на сувата биомаса кај Baycor WP 25 е голем (140.00 mg), а кај Dithane M-45 тој изнесува 20.00 mg.

Според истражувањата, ефикасноста на фунгицидите варира во разни еколошки услови, во зависност од влијанието врз биологијата на патогенот.

Фунгицидите Folicur EW-250 (0.1%), Score 250-EC (0.05%) и Acrobat MZ (0.25%) покажаа најдобри резултати во сузбивањето на патогенот.

Клучни зборови: Alternaria alternata, фунгицид, инхибиција, биологија.

INTRODUCTION

Brown spot is one of the fungal diseases in the Republic of Macedonia which appears each growing season in all tobacco types. Its presence in the oriental tobacco is particularly harmful because it occurs at the end of the season, on the top leaves which have the best quality. According to Rotem (1994), in diseases caused by fungi of the genus Alternaria, the yield may be reduced through: a) reduction of photosynthetic activity and leaf production, without direct infection, b) direct attack by the pathogen, c) reduction of tobacco quality, d) combination of all the activities.

The causing agent of brown spot disease on tobacco - Alternaria alternata reduces the total economic effect of tobacco production through a combination of all mechanisms that affect tobacco yield and quality. It also has an impact on the smoking properties of tobacco raw. As severity of the disease increases, the "good" taste is reduced in favor of the "bad" taste (Lucas, 1975, loc cit Rotem, 1994). Also, the creation of AT toxin by this fungus in tobacco and its persistence in the raw material affects the smokers' health.

The disease is becoming even more important because the intensive way of tobacco production disables the proper performance of agro-technical operations, i.e. the application of preventive measures for protection. The outbreak of the disease is especially affected by the undue harvest of tobacco leaves and excessive irrigation. Because of the above reasons, the application of chemical protection is unavoidable. Therefore, continuous investigations are made on the effectiveness of some active ingredients and fungicides in the control of this pathogenic fungus.

In investigations of Shaeik and Taha (1984), Dithane M 45 applied in concentration of 0.25% appeared to be effective in control of the disease. Nagarajan (2000) recommended the application of Mancozeb, Difenoconazol and Propiconazol for this purpose.

In investigations of Colturato et al. (2009) on the effect of some active ingredients and their combinations on disease intensity and yield increase, all fungicides included in investigation showed a positive effect on reducing the intensity, but trifloxystrobin + propiconazole appeared to be the most effective in increasing the yield.

Fungicides effectiveness in control of A. alternata has been investigated in vitro. Bozukov (2002) studied the biological effect of 15 fungicides in order to determine the most appropriate preparations for tobacco protection from the brown spot disease.

Survilience and Dambrauskiene (2006) reported that active ingredients showed significant inhibitory effect on the development of several species of Alternaria. Issiakhem and Bouznad (2010) found such an effect of Difenoconazole and Chlorthalonil on A. solani and A. alternata. Inhibitory effect on the growth of A. alternata was found in five contact and five systemic fungicides (Chandhary and Patel, 2010).

Mahatabi et al. (2001), in their investigations in vitro and in vivo, found several active ingredients that can be applied in protection of tobacco from brown spot disease. Sometimes, however, certain ambiguities arise between the results obtained in laboratory and in field conditions (Zellner et al., 2011).

Research of biological effect of fungicides in the control of certain pathogen begins with investigation of their impact on its biology as a basic principle for effective protection. Therefore, the aim of this study was to examine the impact of several fungicides on the biology of this pathogenic fungus.

MATERIAL AND METHOD

The selection of fungicides for this investigation was made in accordance with our intention to include higher number of active ingredients known for their protection of tobacco, as well as other substances which proved to be efficient in the control of Alternaria. Some ambiguities in fungicides investigations in field conditions also imposed the need for this research.

The list of systemic and contact fungicides applied on tobacco in recommended concentrations is presented in Table 1.

Table 1. Investigated fungicides

Fungicide	Active ingredient	a.i. content	Concentration, %
Dithane M-45 (WP)	Mancozeb	80 %	0.25
Acrobat MZ (WP)	Dimetomorf +Mancozeb	(9 + 60)%	0.25
Ridomil MZ 72 (WP)	Metalaksil +Mancozeb	(8 +64) %	0.3
Antracol WP –70	Propineb	70 %	0.2
Score 250 -EC	Difenokonazol	250 g/l	0.05
Euparen multi WP 50	Tolyfluanid	50%	0.25
Baycor WP 25	Bitertranol	25 %	0.25
Folicur EW- 250	Tebuconazol	250g/dm^3	0.1
Poliram DF (WG)	Metiram	80 %	0.2

Fungicides effect on conidia germination

Conidia from infected leaves, previously kept in Petri dish on moistened filter paper for 24 hours were used as material for investigation. Water solutions of the fungicides were prepared in appropriate concentrations. The percentage of germinated conidia was estimated by the method of Ko et al. (1975).

Conidia were transferred from lesions with sterile bacteriological needle into the drops of prepared fungicide solutions. They were placed in Van-Tieghem chambers and incubated in a thermostat at 28^0C. The percentage of germinated conidia was estimated after 4-5 hours. The moment when the length of initial hypha was equal to the width was taken as a criterion for germinated conidium.

Five microscopic preparations of each chemical were observed, with 10 visual fields. Conidia suspension in a drop of distilled water was used as a check. The trial was replicated three times and the results present the mean value of the investigations.

The percentage of inhibition of conidia germination was estimated by the Ogbebor and Adekunle formula (2005).

Fungicides effect on pathogen development in solid nutrient media with addition of fungicide

Pure culture of *A. alternata* was used in the trial, obtained by conidia transfer from fresh infected plant material on potato dextrose agar.

The same media was used for monitoring the fungus development in a presence of fungicide. It was prepared in a usual way and fungicides were added after sterilization and cooling to 40-50^0C. The media with appropriate concentration of the fungicide was spread on 110 mm Petri dishes.

3-5 mm^2 fragments of the pure culture (7-10 days old, incubated at 25^oC) were transferred in media with fungicide. The Petri dishes were incubated at the same temperature. The trial was set up in three replications; with every replication five Petri dishes were sown for each variant. The development of the fungus was followed and colony diameter was measured each day, in two opposite directions at right angle. The results given for the 3rd, 5th, 10th and 15th day represent the mean value of the replications. A colony grown in solid media without addition of fungicide was used as a check.

Percentage of reduction of pathogen development in solid and liquid media was estimated according to the formula of Shovan et al. (2008).

Fungicides effect on pathogen development on liquid nutrient media with addition of fungicide

For these investigations, liquid potato dextrose agar was prepared and fungicides were added after sterilization and cooling. 20ml of the media with fungicide was dispensed in Petri dishes and then sowing was performed with fragments of the mycelium using a sterile needle. Five Petri dishes were sown for each variant, and the trial was set up in three replications. In this case, too, the media without fungicide was used as a check. Incubation lasted 15 days at 25^0 C, and it was followed by separation (filtration) of the fungus from the liquid media and drying of the filtrate at 25-28^{0}C up to 3 consecutive measurements with constant value, according to the method of Sarić (1986). The mean value of all replications shows the yield of dry biomass in mg.

RESULTS AND DISCUSSION

All investigated fungicides showed a reducing effect on conidial germination of *A. alternata*. Some fungicides make their impact by inhibiting the development of germ tube, appressorium formation and mycelial growth (Obanor et al., 2005).

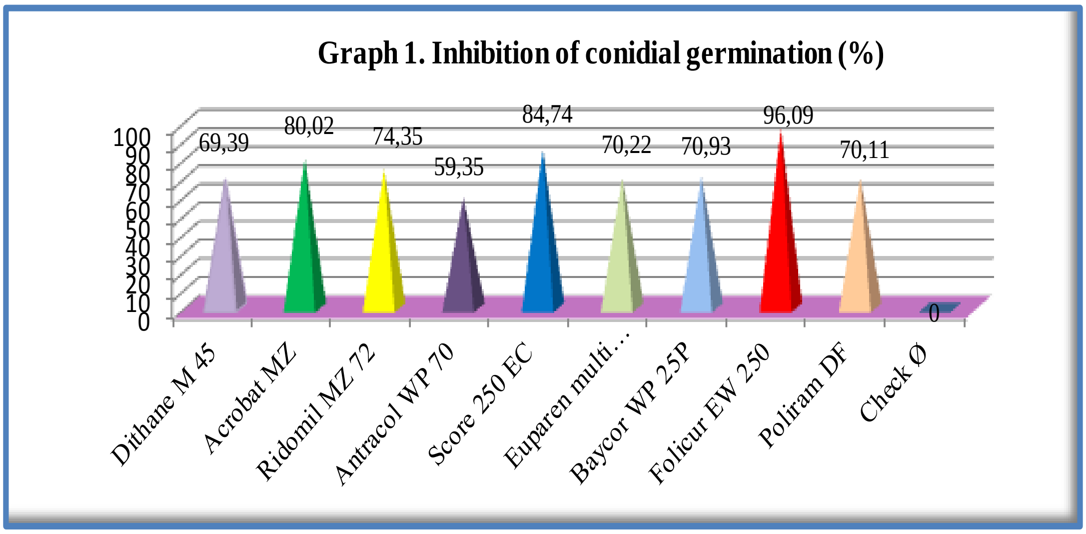
The lowest percentage of germinated conidia was determined with Folicur EW-250, only 2.97%, but also in fungicides Score 250 EC and Acrobat MZ - 11.60% and 15:19, respectively. The highest percentage of germinated conidia was determined with fungicides Dithane M-45 and Antracol WP-70 (Table 2). According to the above data, the lowest percentage of inhibition was obtained with the fungicide Antracol WP-70 - 59.35%. The highest percentage of inhibition of conidia germination was obtained with the fungicide Folicur EW-250 - 96.09%, but also with Score 250-EC and Acrobat MZ (Figure 1).

Table 2. The effect of fungicides on conidia germination

Fungicide	Dithane M-45	Acrobat MZ	Ridomil MZ 72	Antracol WP -70	Score 250 EC	Euparen multi WP 50	Baycor WP25	Folicur EW 250	Poliram DF	Check Ø
Concentration, %	0.25	0.25	0.3	0.2	0.05	0.25	0.25	0.1	0.2	-
Germinated conidia, %	23.27	15.19	19.50	**3.90**	11.60	22.64	22.10	**2.97**	22.72	76.02
Inhibition of conidia germination, %	69.39	80.02	74.35	**59.35**	84.74	70.22	70.93	**96.09**	70.11	-

Similar results were reported by Issiakhem and Bouznad (2010), who also noted that Difenoconazole is effective in conidial germination of *A. alternata* and *A. solani*.

Fungicides differentiated their effectiveness in specified concentration. Those with higher inhibitory activity on germination, even at lower concentrations when the effect on spore germination is slightly reduced, still have an influence which can be seen through the fact that the length of germ tube is significantly reduced (Obanor et al., 2005).



With respect to the development of pathogenic fungus in solid nutrient media with the addition of fungicide, it can be noted that in the initial days of incubation no colony growth was observed with Score 250 EC, as well as with Folicur EW 250, and with the latter it does not appear throughout the entire incubation period (Table 3).

The products start to differentiate with respect to their effect about the fifth day, but in Antracol WP-70, the good development that was observed at the beginning continues up to the end, when the colony size is the largest - 70.90mm. In Ridomil MZ 72, Dithane M-45 and Poliram DF, the colony diameters range from 41.73 to 54.98 mm. These results are similar to those reported by Zellner et al. (2011), in which Poliram WG appeared to have almost no effect on *A. alternata*.

According Chandhary and Patel (2010), Mancozeb shows good results in the inhibition of fungus growth, which is not the case in this type of investigations.

The largest diameter of the colony was observed in the media with Antracol WP-70, i.e. this chemical showed the lowest percentage of inhibition of colony growth - 33.92% (Table 3, Figure 2).

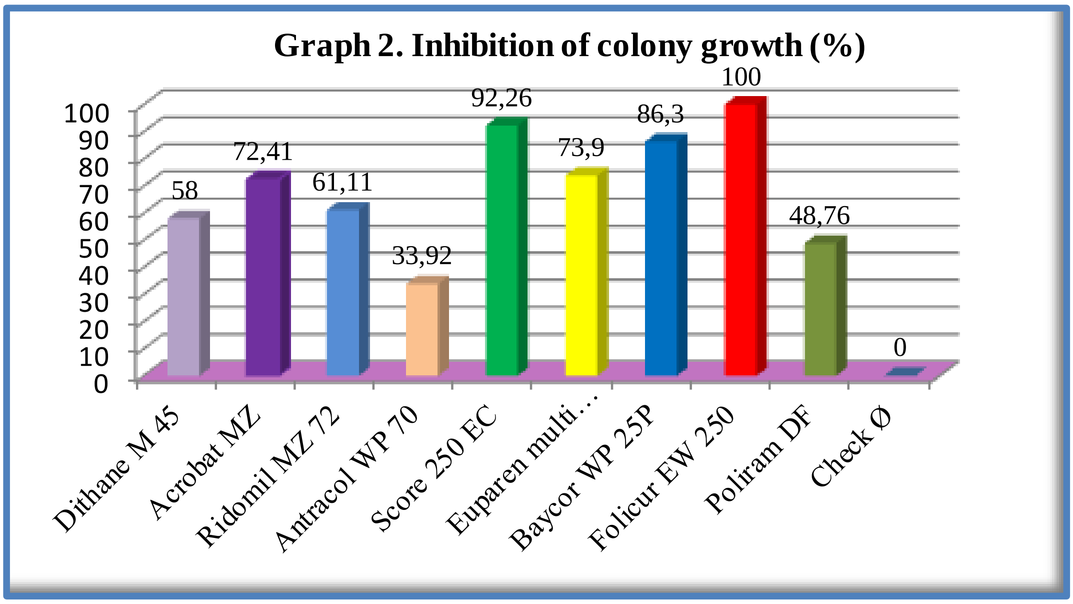
Table 3. The effect of fungicides on colony growth in solid nutrient media

Fungicide	Concentration%	Diameter of the colony (mm)				Inhibition of colony growth %
		3rd day	5th day	10 th day	15 th day	
Dithane M-45	0.25	6.16	8.33	26.50	45.07	58.00
Acrobat MZ	0.25	6.00	8.40	17.70	29.60	72.41
Ridomil MZ 72	0.3	6.00	8.75	24.15	41.73	61.11
Antracol WP-70	0.2	8.50	21.50	42.40	**70.90**	**33.92**
Score 250 EC	0.05	0.00	6.00	6.80	**8.30**	**92.26**
Euparen multi WP 50	0,25	9.30	12.50	21.40	28.00	73.90
BaycorWP25	0.25	6.00	6.00	10.40	14.70	86.30
Folicur EW 250	0.1	0.00	0.00	0.00	**0.00**	**100.00**
Poliram DF	0.2	6.60	10.60	32.23	54.98	48.76
Check Ø	-	27.20	44.02	85.59	107.30	-

Ridomil MZ 72 did not show a significant percentage of inhibition of colony growth (Graph 2, Figure 1). In investigations of Batta (2001), the formulation metalaxyl + mancozeb showed significant curative effect in the disease caused by *A. alternata*. This chemical is also used in the control of other pathogens in tobacco. However, *A. alternata* is less invasive parasite in the complex of fungi that attack tobacco and it is even regarded as secondary parasite (Rotem, 1994). Therefore, certain chemicals may affect other pathogens, which causes an error in determination of the intensity of attack of *A. alternata*. Such explanations are also given by Zellner et al. (2011), for differences in fungicides effect appearing in field and laboratory investigations.

Of the investigated chemicals, only Folicur EW 250 showed 100% inhibition of colony growth (Graph 2, Figure 1 and 2). These results are in accordance with investigations on biological effect of 15 fungicides made by Bozukov (2002), according to which the fungicide effect was recognized in only five chemicals, and the others showed fungistatic and inhibitory effect.

In vitro investigations made by Survilience and Dambrauskiene (2006) revealed that all investigated fungicides have shown satisfactory inhibition of *Alternaria spp*. colonies, reducing them in average 94 to 25% after the 21st day. Folicur EW 250 (Tebuconazol), however, differed by its inhibitory activity which persisted at 71% to 62% even after the 21st day.



Score 250 EC have also achieved high percentage in inhibition of colony growth - 92.26% (Graph 2, Figure 2). These results are in agreement with those of Issiakhem and Bouznad (2010), according to which Difenoconazole is efficient in inhibition of colonial growth of *A. solani* and *A. alternata* and has better effectiveness than the active substance Chlorthalonil.

According to Dahmen and Staub (1992), Difenoconazole shows high effectiveness against the the Ascomycetes, Basidiomycetes and Deuteromycetes classes of fungi.

According to Batta (2001), Difenoconazole together with Cyprodinil + Flodioxonil are the most efficient preventive fungicides in the control of this pathogen in fruits.

In liquid nutrient media with Folicur EW 250, no yield of dry biomass was observed, confirming 100% inhibitory effect of the fungicide in these trials, too. With Acrobat MZ and Score 250 EC it was only 10 mg, or both have shown

95.24% inhibition. These three products showed the best results in the inhibition of dry biomass yield (Table 4, Graph 3).

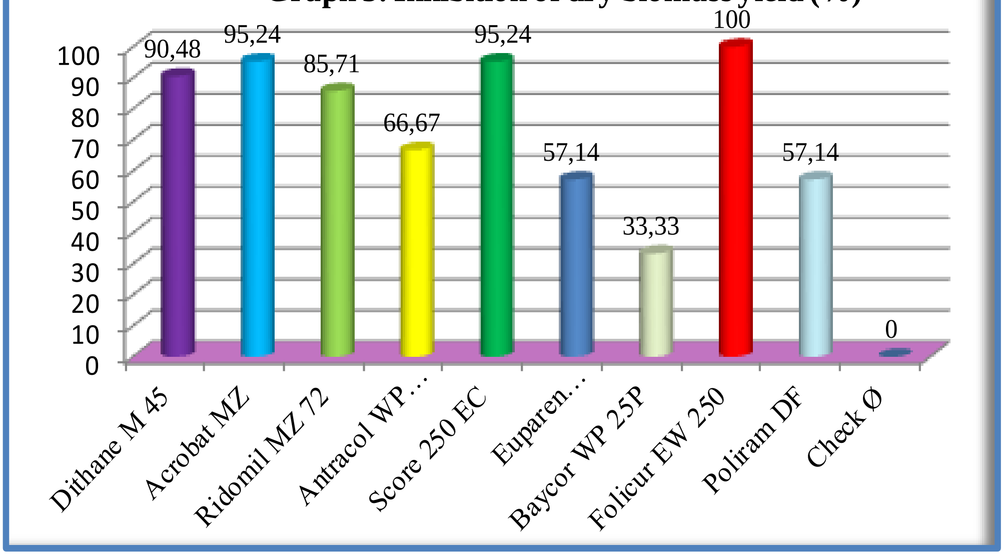
Baycor WP25 showed high efficiency in inhibition of pathogen growth. However, the inhibition of dry biomass yield in liquid nutrient media was only 33.33%, which is the lowest value (Table 3 and 4, Graph 3). Despite the modest results of this product (Table 2) and the small diameter, the colony had a specific look, i.e. it was very thick, in white color, with pronounced aerial growth (Figure 2).

The lowest effectiveness in these trials, besiden Baycor WP 25, were observed with Antracol WP 70, Euparen multi WP 50 and Poliram DF (Graph 3).

With Dithane M-45 fungicide, which showed low inhibitory effect on colony growth, the dry biomass yield was 20mg. It means that in this case the fungicide showed good inhibitory effect, i.e. 90.48% inhibition of dry biomass yield. Similar to this, Chandhary and Patel (2010) reported that percentage of inhibition with mancozeb was 97.80%.

Table 4. The effect of fungicides on dry biomass yield

Fungicide	Dithane M-45	Acrobat MZ	Ridomil MZ 72	Antracol WP -70	Score 250-EC	Euparen multi WP 50	Baycor WP 25	Folicur EW-250	Poliram DF	Check Ø
Concentration, %	0.25	0.25	0.3	0.2	0.05	0.25	0.25	0.1	0.2	
Dry biomass, mg	20.00	10.00	30.00	70.00	10.00	90.00	140.00	**0.00**	**90,00**	210.00
Inhibition of dry biomass yield %	90.48	95.24	85.71	66.67	95.24	57.14	33.33	**100.00**	**57.14**	



Graph 3. Inhibition of dry biomass yield (%)

During investigations, some of the fungicides showed better results in inhibition of colony growth than in conidial germination. The ineffectiveness of certain preparation in protection from conidial germination is due to the fact that it causes higher inhibitory effect on the development of hyphae than on spore germination (Obanor et al., 2005).

Some fungicides (e.g. Acrobat MZ and Dithane M-45), had higher inhibitory impact in liquid than in solid media. Our results are in accordance with findings of Ko et al. (1976), according to which the agar affects the diffusion of fungicides, i.e. it binds to a fungicide, thereby reducing its activity.

Among the investigated fungicides, the following were distinguished by their inhibitory effect on the biology of *A.alternata*: Folicur EW-250 (0.1%) (Tebuconazol), Score 250-EC (0.05%) (Difenoconazole) and Acrobat MZ (0.25%) (Dimetomorf +Mancozeb).

In vitro and in vivo investigations of several active ingredients have shown that Propiconazol, Tebuconazol and Mancozeb in concentration 0.1, 0.1 and 0.2%, respectively, can provide good protection from brown spot disease on tobacco (Mahtabi et al., 2001).

Figure 1. The effect of some fungicides on fungus growth in solid nutrient media

Figure 2. The effect of: (down, left to right) Baycor WP 25, Score 250-EC and Folicur EW 250

CONCLUSIONS

All fungicides investigated had an inhibitory effect on pathogenic fungus A. alternata.

• The best results in the inhibition of conidial germination were obtained with fungicide Folicur EW-250 (0.1%). Good results were also obtained with Score 250-EC (0.05%) and Acrobat MZ (0.25%).

• Folicur EW-250 (0.1%) showed the highest inhibitory effect on pathogen growth. The fungus does not grow in the presence of this product, neither in solid nor in liquid media.

• The lowest yield of dry biomass was obtained with the fungicide Acrobat MZ (0.25%), which showed also good results in solid media.

• Fungicides Baycor WP 25 (0.25%) and Dithane M-45 (0.25%) showed contradictory results in investigations in solid and in liquid media.

• Fuingicides Antracol WP-70 (0.2%) and Poliram EW-250 (0.2%) showed the poorest inhibitory results.

• Fungicides Folicur EW-250 (0.1%), Score 250-EC (0.05%) and Acrobat MZ (0.25%) had the best results in these trials. They can be used in protection of tobacco from brown disease.

SAccording to the results, the effectiveness of fungicides to a particular pathogen varies in different environmental conditions, depending on their impact on biological properties.

REFERENCES

1. Batta Y.A., 2001. Effect of Fungicides and Antagonistic Microorganisms on the Black Fruit Spot Disease on Persimmon. Dirasat, Agricultural Sciences, Vol. 28, No 2&3, p. 165-171.
2. Бозуков Х., 2002. Проучување на биолошкото дејство на некои фунгициди врз Алтернариа алтерната (фриес) Каиссер-причинител на кафената лисна дамкавост кај тутунот. Тутун/Tobacco, Vol 52, No 7-8, 231-233, Прилеп.
3. Chandhary R.F., Patel R.L., 2010. Efficacy of Fungicides against Early Blight of Potato Caused by Alternaria alternata (FR.) Keissler. Journal of Plant Disease Sciences, Volume 5, Issue 2.
4. Colturato A., B., Paulossi T., Venancio W., Wilson S., Furtado E.L., 2009. Efficiency and cost of chemical control of alternaria brown spot. Summa phytopathol, Vol. 35, N0. 3, pp. 210-215.
5. Isiakhem F., Bouznad Z., 2010. *In vitro* evaluation of difenoconazole and chlorthalonil on conidial germination and mycelial growth of *Alternaria alternata* and *A. solani* causal agent of early blight in Algeria. Twelfth EuroBlight workshop Arras (France) 3-6 May, PPO Special Report, No. 14, p. 297-302.
6. Ko W.H., Hsin-hsiung and Kunimoto R.K., 1975. A Simple Method for Determing Efficacy and Weatherability of Fungicides on Foliage.65:1023-1025.
7. Ko W.H., Kliejunas J.T., Shimooka J.T., 1976. Effect of Agar on Inhibition of Spore Germination by Chemicals. Phytopathology 66:363-366.
8. Mahatabi R.A., Zamanizadh H.R., Javid K., 2001. Chemical control of brown spot disease of tobacco caused by Alternaria alternata. CORESTA Meet. Agro-Phyto Groups, Cape Town, abstr. PPOST8.
9. Nagarajan K., 2000. Management Practices for the control of brown spot disease in tobacco, Rajamundry.
10. Obanor F. O., Walter M., Jones E.E., Jaspers M.V., 2005. In vitro effect of fungicides on conidium garmination of *Spilocaea oleagina*, the cause of olive leaf spot. New Zealand Plant Protection 58: 278-282.
11. Ogbebor N., Adekunle A.T., 2005. Rajput N.A., Pathan A.A., Lodhi A.M., Dou D., Liu T., Arain M.S., Rajer F.U., 2012. Inhibition of conidial gereminationn and mycelial growth of *Corynespora cassilicola* (Berk and Curt) of rubber (*Hevea brasiliensis* muell.Arg) using extract of some plants. African Journal of Biotechnology, Vol. 4 (9), pp.996-1000.
12. Rotem J., 1994. The genus Alternaria. APS PRESS. ST.Paul, Minnesota.
13. Sarić M., Kastori R., Petrović M., Stanković Ž., Krstić B., Petrović N., 1986. Praktikum iz fiziologije biljaka. Naučna knjiga, Beograd.
14. Shaeik J., Taha K.H., 1984. Chemical control of brown spot of tobacco in northern Iraq. Indian Phytopathol. 37-4, p. 669-72.

15. Shovan I.R., Bhuiyan M.K.A., Begum J.A., Pervez Z. 2008. In vitro control of *Colletotrichum dematum* causing anthracnose of soybean by fungicides, plant extracts and *Trichoderma harzianum*. Int. J. Sustain. Crop Prod. 3(3): 10-17.
16. Survilience E., Dambrauskiene E., 2006. Effect of different ingredients of fungicides on Alternaria spp. growth in vitro. Agronomy Research 4 (special issue), p. 403-406.
17. Zellner M., Wagner S., Weber B., Hofbauer J., 2011. Effect of fungicides on *Alternaria solani* and *Alternaria alternata*. Bayerische Landersanstalt fur Landwiyschaft, Institut fur Pflanzenschultz.